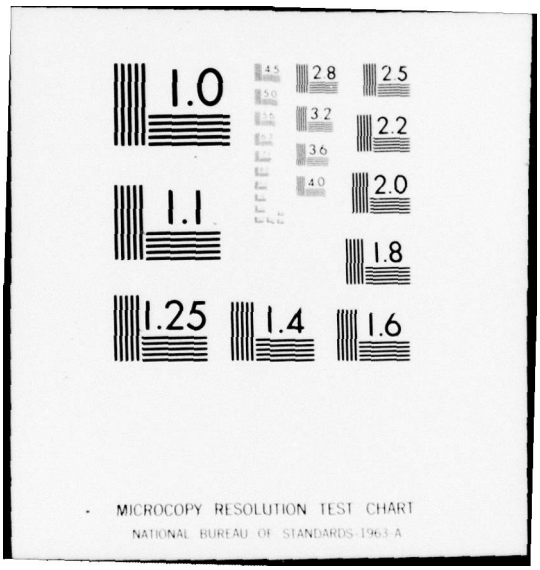
AD-A040 825 CHICAGO MEDICAL SCHOOL ILL DEPT OF PHYSIOLOGY AND BI--ETC F/G 6/19
FINAL PROGRESS REPORT, 1961-1976,(U)
JUN 77 V V GLAVIANO
N00014-67-A-0397-0002
NL

UNCLASSIFIED

1 OF 1
ADA040825

END

DATE
FILMED
7-77


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: N00014-67-A-0397-0002-Final
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): Final Progress Report, 1961-1976
5. TYPE OF REPORT & PERIOD COVERED: Final Report for Shock Project
6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): Dr. Vincent V. Glaviano et al.
8. CONTRACT OR GRANT NUMBER(s): N00014-67-A-0397-0002

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Health Sciences
The Chicago Medical School
2020 W. Ogden Avenue., Chicago, Illinois 60612

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 201-144

11. CONTROLLING OFFICE NAME AND ADDRESS
Department of the Navy
Office of Naval Research
Arlington, Virginia 22217

12. REPORT DATE: June 16, 1977
13. NUMBER OF PAGES: 11

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
Office of Naval Research Branch Office
Chicago
536 South Clark Street
Chicago, Illinois 60605

15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Distribution is unlimited.

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

16 Jun 77

DDC
RECEIVED
JUN 21 1977
C

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
13p.

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Hemorrhagic shock, burn shock, myocardial catecholamines, histamine, lactate dehydrogenase, myocardial depressant factor, glycogenolysis, phosphorylase activity, propranolol, electrolytes, stellate ganglion stimulation, carotid occlusion, free fatty acids, prostaglandin E_1, subcellular lipids, carbachol, sympathetic-parasympathetic interaction

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
This laboratory has completed its long standing research objective in determining the role of the heart in hemorrhagic or burn shock. Metabolic alterations in the heart during hemorrhagic shock were determined from changes in catecholamines, electrolytes, carbohydrates and enzymes. Myocardial depressant factor was not found to contribute to the terminal shock state. Phenoxybenzamine appeared to have a beneficial effect on renal function by enhancing K^+ excretion. Metabolic alterations in burn shock seem to have arisen from coronary insufficiency as determined from changes in catecholamines, histamine, lipids, lipases and LDH activity. A hepatic metabolism study showed that the liver likely plays a major role in the pathogenesis of burn shock. In addition, norepinephrine, propranolol, prostaglandin E_1, and carbachol were used to study changes in myocardial cyclic nucleotides, lipids, lipid turnover and sympathetic-parasympathetic nervous system interaction.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

408 536

U.S. Navy Department Office of Naval Research

Final Progress Report

1961 - 1976

of

Contract N00014-67-A-0397-0002

Report prepared by: Vincent V. Glaviano, Ph.D.

Professor and Chairman of Physiology and Biophysics

University of Health Sciences/The Chicago Medical School

2020 West Ogden Avenue

Chicago, Illinois 60612

June 16, 1977

The investigations during the period of this contract have covered studies in hemorrhagic and burn shock as well as myocardial metabolism and hemodynamics.

In regard to the studies in hemorrhagic shock, severe depletion of endogenous norepinephrine was observed in the brain, heart, liver, and spleen of albino rabbits in which hemorrhagic shock had been induced. On the other hand, the epinephrine content of these tissues was significantly elevated above the levels in tissues of control animals. The norepinephrine and epinephrine levels of skeletal muscle in shocked animals remained unaffected. Probably the rise in the concentrations of epinephrine in tissue stems from the high adrenal output of this hormone in hemorrhagic shock (4). However, our experimental data do not give any indication of the mechanism of norepinephrine depletion. Experiments for comparing the rate of synthesis with the rate of utilization of norepinephrine would explain the lowering of levels of this catecholamine in tissue of the shocked animal.

Electrolyte and water content were determined in left ventricular muscle in control dogs and dogs subjected to irreversible hemorrhagic shock. In shock, myocardial potassium increased from 39.6 to 47.7 mEq/100 g fat-free dry tissue, while sodium decreased from 16.4 to 13.2 mEq/100 g fat-free dry tissue. Water content, as well as levels of magnesium, calcium, and chloride were not significantly altered. Changes of intracellular potassium, sodium, magnesium, and calcium were within limits expected from Gibbs-Donnan equilibrium relationships. However, interstitial potassium and magnesium were markedly elevated from 4.56 to 7.52 mEq/kg and from 1.29 to 2.54 mEq/kg, respectively. As a result of the increase in interstitial potassium, the calculated average resting membrane potential differed by 11 mv from control dogs. The observed alterations in myocardial electrolytes in our shock experiments are not similar to those generally encountered in heart failure. On this basis, a cardiac factor may not be a major cause of the irreversible state of hemorrhagic shock.

In a comparison of myocardial K, Na, Cl and H_2O in normal dogs and dogs in hemorrhagic shock, levels of K were found significantly elevated. Although Na concentrations declined, H_2O content did not change from controls. Administration of l-norepinephrine to dogs in normovolemic shock caused no change in K and H_2O while Na and Cl levels returned to control. Analysis of myocardial K distribution showed that intracellular/interstitial ratio of this cation declined from 33 to 21 in shock. Norepinephrine treatment of these animals raised K ratio to 29, a change that primarily reflected the increase which this pressor amine causes in intracellular K.

Cardiac responses to electrical stimulation of the right or left stellate ganglion were recorded from 16 open-chest anesthetized dogs in hemorrhagic shock. Shock was induced by bleeding the animals to a mean blood pressure of 40 mm Hg. This level of pressure was maintained for 4 hr, during which time blood pressure, heart rate, force of myocardial contraction, and intraventricular pressures were recorded. Stimulation of the stellate ganglion for 15-40 sec every 30 min after hemorrhage showed a gradual decrease in these parameters to levels below control. The reinfusion of blood and the infusion of exogenous l-norepinephrine did not restore an increase in force of cardiac contraction to stellate stimulation. Myocardial epinephrine and norepinephrine levels in shock were found not to differ from those in 14 normal dog hearts. In contrast to almost complete myocardial refractoriness to stellate stimulation in hemorrhagic shock,

stimulation of the vagus nerve elicited bradycardia and eventual cardiac arrest. The decrease observed in force of cardiac contraction to stimulation of the stellate ganglion in hemorrhagic shock may be due to depletion of norepinephrine stores in the heart.

Anesthetized dogs atropinized or bilaterally vagotomized were subjected to bilateral common carotid artery occlusion to determine the functional status of the carotid sinus reflex before and during various stages of hemorrhagic shock. In hypotensive and post-infusion stages of shock, the response in cardiac output before and during carotid occlusion showed no changes; however, arterial pressure and TPR were invariably found to increase. The results of this study indicate that, regardless of the stage of shock, the carotid sinus reflex can be elicited by bilateral common carotid occlusion. The response in total peripheral resistance was found to be greater in control and oligemia than that observed in the post-infusion irreversible stage of shock. In the terminal stage, peripheral vascular failure was noted and postulated to be due to an accumulation of tissue metabolites surrounding the peripheral vasculature.

The concentration of metabolic substrates in cardiac muscle and in arterial and coronary venous blood was determined in control dogs and dogs in hemorrhagic shock. Levels of lactate, pyruvate, glycogen, and phosphorylase enzyme in left ventricular muscle were compared between the two groups of animals. Anoxia of the myocardium, often suggested to occur in hemorrhagic shock, was not substantiated from the increase in glycogen and decrease of phosphorylase activity in cardiac muscle of dogs in shock. On the other hand, the significant elevation in myocardial lactate does suggest the occurrence of some degree of anaerobic metabolism. In the control group, arterial and coronary sinus blood lactate and pyruvate were observed to be lower than the level of the two substrates in cardiac muscle. Although myocardial oxygen uptake in hemorrhagic shock did not differ from control, concentration of lactate and pyruvate in blood rose to levels higher than those found in the myocardium. Although cardiac anoxia in hemorrhagic shock can be postulated from the increase in myocardial lactate, the rise in glycogen and decrease in phosphorylase activity, accompanied by no change in myocardial oxygen uptake, are considered contrary to this postulate.

Myocardial performance was evaluated in nine pancreatectomized and 12 nonpancreatectomized dogs by measuring left ventricular pressure (LVP), maximal dP/dt), left ventricular end-diastolic pressure (LVEDP), pulmonary arterial pressure (PAP), aortic pressure (AoP), and lead II of the electrocardiogram during standardized hemorrhagic shock. Cardiac output (CO) and hematocrit were determined before hemorrhage, after 4 h of oligemia, and when postinfusion mean blood pressure declined to 60 mmHg. Left ventricular function curves were obtained, by varying preload, in control dogs and 2 h after reinfusion of the shed blood in those dogs subjected to shock. Both groups of dogs showed identical responses to the shock procedure. In the immediate postinfusion period, LVP, max dP/dt, LVEDP, and mean blood pressure returned to near-control values, while PAP was significantly elevated. The post infusion decline (after 60-90 min) in AoP was accompanied by a similar reduction in LVEDP. Left ventricular performance in hemorrhagic shock did not differ significantly from that seen in control dogs. In addition, there was no electrocardiographic indication of myocardial ischemia. The data indicate that terminal hemorrhagic shock need not be accompanied by myocardial depression whether or not the pancreas is intact.

This study was undertaken to determine if alpha-adrenergic blockade would reverse the altered kidney function in hemorrhagic shock. Urinary excretion of Na^+, K^+, and total solute were studied in 10 anesthetized dogs before and after administration of phenoxybenzamine (PBZ) in shock. Five of the 10 dogs were used to evaluate the effect of shock on renal hemodynamics. Another group of 5 dogs were used as controls. Normovolemic

shock caused a reduction in renal blood flow (RBF) from 9.0±1 ml/min/kg to 6.0±0.6 ml/min/kg, and an increase in renal vascular resistance (RVR $P<.05$). When compared to the untreated shock animals, phenoxybenzamine caused an 83 percent increase in RBF and a 53 percent decrease in RVR. Phenoxybenzamine caused a 10-fold increase in urinary NA+ concentration in the control animals, but had no effect on level of urinary K+. Neither urinary K+ nor urinary Na+ was affected by PBZ in the shock group. Although the excretion rate for Na+ was increased several fold in the untreated shocked animals when compared to controls (14.0±3 μEq/min vs 1.7 μEq/min) the excretion rates for K+ ($P<.05$) and total osmotically active substances ($P<.01$) diminished. The beneficial effect of phenoxybenzamine on renal function in the shocked animal appears to involve an enhanced excretion of potassium.

In a study of the glycolytic metabolism of the dog's heart during hemorrhagic shock, levels of lactate in cardiac muscle could not be accounted for by the concentration of lactate or pyruvate in arterial or coronary sinus blood. On the basis of these observations, the distribution and gradient of lactate between blood and cardiac muscle was further investigated in the normal dog myocardium. The concept that blood lactate is in equilibrium with myocardial concentrations of this substrate could not be demonstrated in the anesthetized dog. A factor questioning the existence of an equilibrium was the distribution of lactate found in the walls of the heart. Levels in right and left atria were markedly different from those found in the base, midportion and apex of the right and left ventricles. Although administration of sodium lactate by vein raised arterial lactate almost 6 times control, right and left ventricular muscle concentrations remained unchanged. The linear relationship observed between the duration of myocardial anoxia and lactate production by cardiac muscle made possible the calculation of the *in vivo* myocardial lactate level, a value significantly higher than the level present in arterial blood. The entry of lactate into the myocardial cell may therefore be governed by a mechanism of active transport rather than simple diffusion.

The lactate level of blood entering and leaving the lungs during electrical stimulation of its sympathetic innervation was studied in 16 dogs anesthetized with chloralose. The production of lactate by the lungs was determined from the product of the A-V blood difference across the lungs and the cardiac output. Blood samples for analysis were taken from catheters placed in the right and left ventricles. Cardiac output was determined from the dye-dilution method . In the control period, the lactate release from the lungs averaged 27.0 mg/min, whereas stimulation for 10 min caused an increase to an average of 133.9 mg/min. Fifteen to thirty minutes after stimulation, formation of lactate decreased to 39.06 mg/min. Levels of lactate in blood-free lung tissue samples were determined before, during, and after sympathetic nervous stimulation. Lactate levels in the control period averaged 3.86 mg/100 g of lung tissue, whereas during stimulation the level rose to 6.57 mg/100 g.

Metabolic changes observed in response to hemmorhagic shock suggested that similar changes might be observed in response to burn shock. Consequently we began our investigations in burn shock with measurements of myocardial catecholamine levels during the first few hours of acute burn shock.

The norepinephrine and epinephrine content of ventricular cardiac muscle and spleen were analyzed in 2 groups of anesthetized white rats. One group served as controls, while the other group was subjected to burn shock induced by immersing the lower 1/2 of the body for 90 sec in water heated to 90° C. Myocardial norepinephrine and epinephrine levels of control rats averaged 0.74 ± 0.15 and 0.07 ± 0.06 μg/gm, respectively. In burn shock, norepinephrine decreased markedly to 0.26 ± 0.20 μg/gm ($p<.001$), while epinephrine rose to 0.24 ± 0.07 μg/gm ($p<.003$). On the other hand, splenic norepinephrine rose from 0.55 ± 0.15 to 1.02 ± 0.25 μg/gm ($p<.05$), while epinephrine content

declined from 0.36 ± 0.12 to 0.09 ± 0.06 μg/gm ($p < .003$) in burn shock. Excessive sympathetic nervous system stimulation, or the formation of toxic substances may be involved in reducing norepinephrine stores of the myocardium in burn shock. Unlike the depletion of splenic norepinephrine in hemorrhagic shock, the norepinephrine content of the spleen rose. This finding together with postmortem observations of a soft and relaxed spleen would suggest a different role than that observed for this organ in hemorrhagic shock.

In anesthetized dogs in burn shock for 4 hr, the rate of rise in arteriovenous difference of histamine across the lungs was measured from blood entering and leaving the lungs via the right and left ventricles of the heart, respectively. A maximal uptake of blood histamine by the lungs was attained within 15 min following burn injury. In subsequent periods following the burn injury, the pulmonary arteriovenous difference declined while arterial and venous histamine levels remained elevated until the experiment was terminated. Of the hemodynamic alterations recorded, the most significant change was the immediate decrease in cardiac output following burn trauma. The uptake and storage of histamine by the lungs may be related to pulmonary complications observed in patients in burn shock.

The histamine levels of pulmonary arterial and venous blood in dogs subjected to thermal injury were measured, and a comparison was made between the dogs that did and those that did not survive a 240 min period of burn shock. Thermal injury produced an elevated histamine level in pulmonary arterial and venous blood in the two groups, but a marked difference was found in the amount and the rate of rise in blood histamine, especially in the arterial blood between the dogs that succumbed and those which survived acute burn injury. The measurement of the pulmonary arteriovenous difference of histamine is suggested as a means of estimating the severity of burn injury.

In anesthetized dogs subjected to 4 hr of shock from infrared burns, lipolytic activity of cardiac muscle was found significantly below the activity determined in normal heart muscle. Myocardial homogenates from control and burned dogs responded to the addition of epinephrine with an increase in rate of lipolysis after incubation for 10 min. Incubation of the media for 1 hr showed a further increase in lipolysis, although no difference was noted in activity between epinephrine-treated and untreated homogenates from either the control or burn group of dogs. The addition of inhibitors or activators to the lipolytic mixture prepared from normal hearts suggested that cardiac lipolytic activity results from both an epinephrine-sensitive lipase and a lipoprotein lipase. The epinephrine-sensitive lipase could not be classified as a monoglyceride lipase, since epinephrine as well as ATP and cyclic AMP failed to increase the rate of lipolysis in cardiac muscle homogenates containing monopalmitin substituted for Ediol as a substrate.

Myocardial norepinephrine and epinephrine levels in burned dogs were not different from those found in control dogs. The absence of a change in these cardiac catecholamines leaves the possibility that other humoral or toxic substances may be responsible for altering myocardial lipolysis in burn shock.

The rate of change in histamine in the skin and blood was determined before and after subjecting anesthetized dogs to a skin burn by infra-red radiation. A reduction in histamine averaging 41% of the control value was observed to decrease maximally within 60 min after the burn injury. In the same period of time a maximal increase in blood histamine occurred of 84% above control. The rise in blood histamine would suggest that in large part the blood level in burn injury arises from histamine released from the skin. The data would also suggest that skin histamine is only partially depleted by thermal injury.

Anesthetized dogs subjected to a standardized cutaneous burn injury covering 35% of their surface area showed a marked increase in total plasma LDH activity. The increase in LDH activity was accompanied, as determined in another group of burned dogs, by a significant decrease in cardiac output and an increase in total peripheral resistance and hematocrit. In control dogs, plasma LDH activity showed little or no change during a 4-hr period, while burned dogs increased their plasma LDH activity from 44.7 to 137.0 units/ml. The negative myocardial uptake of LDH indicated that cardiac muscle in burned dogs released LDH into the circulation. In the absence of burn trauma, dogs subjected to the same experimental procedures had no significant efflux of LDH from the heart. The release of myocardial LDH in the burned animal may possibly be related to the action of a burn toxin, a state of myocardial anoxia or the presence of an underlying injury to the heart.

Myocardial hemodynamics and metabolism were studied in anesthetized dogs subjected to a full thickness skin burn covering 35% of the body surface area. As compared with non-burned dogs, dogs in burn shock for 4 hours had a more significant decrease in cardiac output and a greater increase in peripheral resistance and hematocrit. The decreases found in left ventricular *dp/dt* and coronary blood flow were accompanied by insignificant changes in central venous pressure and left ventricular end diastolic pressure. Although myocardial extraction of lactate and free fatty acids did not change, glucose extraction increased while pyruvate decreased in the postburn period. The reduced coronary blood flow, rather than a change in the myocardial extraction of oxygen after the burn, was found to be mainly responsible for the decrease in left ventricular oxygen uptake. In the absence of clinical cardiac failure, depressed cardiac contractility was attributed to hypoxia as characterized by the decrease in oxygen uptake and the alterations found in myocardial carbohydrate metabolism after the burn injury.

To study the changes produced by burn trauma in hepatic metabolism, liver blood flow and the uptake of oxygen and lactate were measured in 12 anesthetized mongrel dogs. Radio-opaque catheters were placed under fluoroscopic guidance in the ascending aorta and the hepatic vein. From an abdominal incision a catheter was passed into the portal vein from a mesenteric vessel. Burn shock was induced by subjecting the ventral surface of the abdomen and lower extremities to infra-red heat for 6 min, which resulted in a full thickness skin burn covering 30-35% of the animal's surface area. Four hours after burn injury, the dye dilution method for cardiac output showed a significant decrease from 2.02 (control) to 0.44 L/min, while the arterial hematocrit rose from 47 to 59%. As compared to control, changes in mean arterial blood pressure and heart rate were minimal. Hepatic blood flow as determined by the method of Banaszak decreased from 57 to 22 ml/min/kg. The burn injury caused hepatic oxygen and lactate uptake to decline from 2.03 to 1.22 ml/min/kg and from 9.0 to -0.3 μM/min/kg respectively. From the significant decreases that were observed in hepatic blood flow, oxygen consumption and lactate extraction, the liver most likely plays a major role in the pathogenesis of burn shock.

During the studies on hemodynamic and burn shock it was felt that myocardial metabolic changes would be better understood if the effects of natural and/or synthetic agonists and antagonists on myocardial performance were evaluated.

The myocardial metabolic effects of the *beta* adrenergic blocking drug, *dl*-propranolol, were studied in 19 open-chest anesthetized mongrel dogs in which arterial and left intraventricular pressures, heart rate, coronary blood flow and velocity of left ventricular pressure development (*dp/dt*) were measured. Myocardial uptake of glucose, lactate, pyruvate and free fatty acids (FFA) was determined during a control period and 10 min after the administration of propranolol (1 mg/kg). A significant decrease in the uptake of FFA (48.3%) occurred during *beta* receptor blockade, whereas glucose,

lactate and pyruvate uptake did not change from control. To determine whether the decline in uptake of FFA after propranolol was due to a reduction in myocardial energy requirements or to a specific metabolic blockade, heart rate was elevated with pacing electrodes on the left atrium by approximately 50 beats/min. In control dogs, atrial tachycardia caused a marked increase in FFA uptake with no significant change in the uptake of carbohydrates. In propranolol-treated dogs, atrial tachycardia increased the uptake of glucose (54.1%) and lactate (13.9%), whereas FFA uptake decreased by 19.4%. These results demonstrate that propranolol interferes specifically with the myocardial uptake of FFA.

Cardiac beta-adrenergic receptors were stimulated by the infusion of 0.02 μg/min/kg of norepinephrine (NE) into the left circumflex coronary artery in 20 anesthetized, bilaterally vagotomized open chest dogs. NE increased myocardial extraction ratio and uptake of glucose, lactate and plasma unesterified fatty acids (FFA). The increase in metabolism was accompanied by a slight rise in peak intraventricular pressure and a significant increase in dp/dt. Mean blood pressure, coronary artery perfusion pressure, coronary flow and coronary vascular resistance were not altered. In 10 of 20 dogs treated with the beta-adrenergic receptor blocking drug, dl-propranolol, the intracoronary infusion of NE caused moderate decreases in the uptake of lactate and pyruvate, while that of glucose was not significantly altered. FFA uptake was markedly decreased from 12.7 ± 3.3 to 1.0 ± 2.3 μEq/min/100 g while the extraction ratio declined 58% from control. Myocardial glycogen concentration in 8 control dogs was found to be not significantly different from cardiac glycogen levels in dogs infused with NE before and after beta-receptor blockade. These data indicated that the myocardial uptake of FFA in dogs pretreated with propranolol was not stimulated by subsequent administration of NE, suggesting that FFA utilization by the normal heart is closely associated with the cardiac beta-adrenergic receptor.

In anesthetized dogs intracoronary administration of prostaglandin E_1 (PGE_1, 0.5 μg/kg per min) for 10 min caused a decline in arterial levels, myocardial extraction ratio, and uptake of FFA. The decrease was accompanied by an elevation in arterial levels, myocardial extraction ratio, and uptake of glucose. Cardiac muscle TGFA rose while both myocardial oxygen uptake and coronary blood flow decreased. In another group of dogs, a lower dose of PGE_1 (.05 μg/kg per min) had a similar but less pronounced effect on myocardial FFA metabolism. PGE_1 added to cardiac muscle homogenates from control dogs depressed lipolytic activity. Gas chromatographic analysis of individual FFA in arterial and coronary venous blood showed PGE_1 to have a general lowering effect on all acids analyzed. Hemodynamic changes recorded before, during, and after the administration of PGE_1 showed mean blood pressure to decrease during the infusion while coronary flow, which initially increased, gradually declined to a level below control. Changes in heart rate, myocardial contractile force, and coronary vascular resistance were minimal. The action of PGE_1 in inhibiting myocardial lipolysis was found to resemble the antilipolytic effects of PGE_1 on adipose tissue and the previously reported action of the β-adrenergic blocking compound, dl-propranolol on the heart. The results suggest that the antilipolytic properties of PGE_1 may stem from a lowering of myocardial cyclic AMP.

Myocardial subcellular alterations in free fatty acids (FFA) and triglycerides (TGFA) were measured in left ventricular muscle from open-chest mongrel dogs after the administration of propranolol (1 mg/kg i.v.) and norepinephrine (NE, 0.2 μg/kg/min i.v.). In one group of experiments (group I), FFA and TGFA levels were determined in nuclear, mitochondrial, microsomal and supernatant fractions in control dogs and in dogs after the administration of NE or propranolol. In this group of experiments, propranolol elevated the TGFA concentration in mitochondrial, microsomal and supernatant fractions

from control whereas the levels of FFA in the same fractions declined, with the most significant changes occurring in the supernatant fraction. These results confirmed earlier reports from this laboratory that propranolol may block myocardial FFA uptake by inhibiting intracellular TGFA degradation. NE was found to have no significant effect on the level (pool size) of FFA and TGFA in subcellular fractions of the myocardial muscle cell. In another group of experiments (group II), Na-palmitate-1-^{14}C (50 μc) was infused into the left circumflex coronary artery of dogs treated with propranolol or NE. Subcellular fractions were isolated and radioactivity of FFA, TGFA and phospholipids from these fractions was determined by liquid scintillation. Propranolol significantly increased activity of TGFA in the mitochondrial, microsomal and supernatant fractions which confirmed the inhibition of TGFA degradation found in group I with propranolol. However, NE increased the activity of TGFA in the mitochondrial, microsomal and supernatant fractions, indicating that the turnover of FFA through the TGFA moiety was increased, whereas its total intracellular level remained unchanged as observed in group I experiments. In response to both propranolol and NE, radioactivity in the FFA pool was reduced only in the supernatant fractions whereas little change was observed in phospholipid activity. The results of these experiments support the hypothesis that subcellular fractions containing pools of TGFA may have a role, especially in the supernatant fraction, in determining the myocardial uptake of FFA from arterial blood entering the heart.

The effects of a 6 min infusion of carbamylcholine chloride (carbachol, 10 μg/min) on arterial plasma levels of free fatty acids (FFA), glucose, arterial blood pressure and heart rate, were studied in anesthetized dogs. Carbachol significantly increased the arterial plasma levels of free fatty acids (FFA) and glucose by 42 and 35 percent respectively. Functional adrenalectomy, followed by the infusion of carbachol, lowered blood pressure and heart rate while abolishing the increase in FFA and glucose. Muscarinic blockade induced by atropine did not affect plasma levels of FFA, although a significant increase in glucose concentration was observed which likely reflected an unmasking of sympathetic nervous activity. Atropine blockade followed by carbachol infusion inhibited the increase in plasma FFA and glucose. In another group of dogs, nicotinic blockade with hexamethonium produced a significant decrease in FFA with little change in glucose. Subsequent infusion of carbachol in these dogs did not alter plasma FFA concentration, although an increase was observed in plasma glucose. A major action of carbachol in altering plasma FFA and glucose was found to be mediated primarily through stimulation of the adrenals.

In open-chest dogs anesthetized with sodium pentobarbital, acetylcholine (ACh, $5 \times 10^{-5}M$) infused into the left circumflex coronary artery caused an increase in coronary flow and a decrease in myocardial O_2 extraction ratio ($P<.01$) and uptake ($P<.05$). Heart rate and mean arterial pressure were not altered, although left ventricular dP/dt declined from 2,037 ± 205 to 1,873 ± 194 mmHg/s ($P<.02$). Intracoronary administration of norepinephrine (NE, $2.4 \times 10^{-6}M$) caused an increase in myocardial O_2 uptake ($P<.02$); simultaneous infusion of both NE and ACh caused a decline in O_2 extraction ratio ($P<.01$) and uptake ($P<.05$). Myocardial adenylate cyclase activity in response to ACh was not altered significantly from a control level of 188 ± 22 pmol of ^{14}C-labeled cyclic AMP/mg protein per 10 min. Norepinephrine alone elevated adenylate cyclase activity to 401 ± 45 pmol (^{14}C)cyclic AMP/mg protein per 10 min ($P<.01$). However, with simultaneous infusion of both NE and ACh, adenylate cyclase returned to control levels. Although ACh alone did not alter myocardial hormone-sensitive lipase activity, NE elevated lipolytic activity from 8.1 ± .7 to 13.2 ± 1.8 μeq free fatty acid (FFA)/g per 30 min ($P<.05$). The administration of both ACh and NE returned lipase activity to nearly control levels. Myocardial uptake of FFA increased significantly during ACh infusion alone ($P<.05$) and during NE infusion alone ($P<.05$). However, when NE and ACh

were administered together, a decline in FFA uptake was observed ($P<.02$). These data indicated that the effects of ACh on cardiac metabolism are minimal, with the decline in myocardial O_2 uptake of ACh primarily reflecting the decrease in contractility. On the other hand, antagonism of ACh on NE-stimulated myocardial lipid metabolism appears to involve activity of the adenylate cyclase system.

BIBLIOGRAPHY

ABSTRACTS

1. Coleman, B. and V. V. GLAVIANO. Myocardial composition in irreversible hemorrhagic shock. Fed. Proc. 21:119, 1962.
2. GLAVIANO, V. V. and B. Coleman. Alterations of myocardial tissue constituents in irreversible hemorrhagic shock. Proc. Int. Union Physiol. Sci. (Leiden) 2:682, 1962.
3. Coleman, B. and V. V. GLAVIANO. Effects of norepinephrine on electrolytes and water content of cardiac muscle in hemorrhagic shock. Fed. Proc. 22:288, 1963.
4. Doersching, J., B. Coleman and V. V. GLAVIANO. Glycolytic metabolism of the heart in hemorrhagic shock. Physiologist 6:169, 1963.
5. Moran, J., J. Doersching and V. V. GLAVIANO. Mechanism of hypotension in acute spinal shock. Fed. Proc. 23:415, 1964.
6. GLAVIANO, V.V., A. Appel and J. Doersching. Distribution and gradient of lactate between blood and heart muscle. Physiologist 7:143, 1964.
7. Traxler, J. and V. V. GLAVIANO. Evidence contrary to the concepts of cardiac failure in hemorrhagic shock. Physiologist 8:290, 1965.
8. GLAVIANO, V.V., S. Yo and V. Nemickas. Cardiodynamic responses to bilateral carotid artery occlusion in hemorrhagic shock. Proc. Int. Union Physiol. Sci. XXIII, Tokyo, Japan, 1965.
9. GLAVIANO, V.V., S. Yo and T. Masters. Pulmonary tissue production of lactate during sympathetic stimulation. Fed. Proc. 25:693, 1966.
10. Masters, T.N. and V.V.GLAVIANO. The effects of propranolol on myocardial carbohydrate and fatty acid metabolism. Physiologist 10:245, 1967.
11. Guerrero, M.J. and V.V.GLAVIANO. Hemodynamic effects of blood and dextran infusions in dibenzyline treated dogs in hemorrhagic shock. Physiologist 10:189, 1967.
12. GLAVIANO, V.V. and M. Guerrero. Skeletal muscle metabolism in dibenzyline-treated dogs in hemorrhagic shock. Physiologist 10:182, 1967.
13. GLAVIANO, V.V. and T. Masters. The effects of intracoronary norepinephrine on cardiac metabolism before and after beta-adrenergic blockade. Fed. Proc. 26:771, 1967.
14. Kaplan, A.D. and V.V.GLAVIANO. Myocardial electrolyte changes, before and during ventricular fibrillation and following electrical defibrillation. Texas Report on Biol. and Med. 26:430, 1968.
15. GLAVIANO, V.V. and T. Masters. Inhibitory action of Prostaglandin E_1 on myocardial lipolysis. Circulation 34:83, 1968.
16. Cova, R., A. Suzuki and V.V.GLAVIANO. Lung tissue levels and pulmonary arteriovenous blood difference of histamine in burn shock. Fed. Proc. 27:448, 1968.
17. Masters, T.N. and V.V.GLAVIANO. Alterations in free fatty acids and triglycerides in subcellular fractions of cardiac muscle during B-adrenergic receptor stimulation and blockade. Fed. Proc. 28:280, 1969.
18. Masters, T.N. and V.V.GLAVIANO. Myocardial subcellular distribution of Na-Palmitate-1-^{14}C and the effects of dibutyryl c-AMP on B-adrenergic receptor activity and fatty acid metabolism. Fed. Proc. 29:449, 1970.
19. GLAVIANO, V.V. and T. N. Masters. Cardiac muscle lipolytic activity and catecholamines in burn shock. Fed. Proc. 30:388, 1971.
20. Masters, T.N. and V.V.GLAVIANO. Myocardial metabolic and hemodynamic effects of dibutyryl-c-AMP before and during B-adrenergic receptor blockade. Fed. Proc. 30:459, 1971.
21. Sukowski, E. and V.V.GLAVIANO. Effects of acetylcholine on triglyceride metabolism of the isolated rat heart. Fed. Proc. 31:351, 1972.
22. GLAVIANO, V.V., A. Okamoto, M. Kaye and Deets. Arterial levels and myocardial extraction of metabolic substrates in acute burn trauma. Physiologist 15:147, 1972.

23. Coleman, B., J. E. Kallal, L. P. Feigen and V.V.GLAVIANO. Myocardial performance in irreversible hemorrhagic shock. Physiologist 18:285, 1973.
24. Goldberg, J. M. and V.V.GLAVIANO. The action of carbamylcholine (carbachol) on plasma levels of free fatty acids, triglycerides and glucose. Fed. Proc. 32:742, 1973.
25. GLAVIANO, V.V., J. M. Goldberg and M. Pindok. Acetylcholine inhibition of norepinephrine stimulation of myocardial lipid metabolism. Fed. Proc. 32:404, 1973.
26. Goldberg, J.M., M. Pindok and V.V.GLAVIANO. The dissociation of myocardial inotropism from metabolism by carbachol in dogs subjected to cardiopulmonary bypass. Physiologist 16:324, 1973.
27. Feigen, L.P., J. Shanes, B. Coleman and V.V.GLAVIANO. The effect of alpha-adrenergic blockade on renal function during acute hemorrhagic shock. Fed. Proc.33:318, 1974.
28. Wallick, D., C. Aravanis, M. Pindok and V.V.GLAVIANO. Effect of carbachol on lipid levels in the non-working canine myocardium. Physiologist 17:353, 1974.
29. Barandiaran, L.F., D. Wallick and V.V.GLAVIANO. Alterations in flow, oxygen consumption and lactate extraction of the liver in burn shock. Fed.Proc.34:352, 1975.

PAPERS

1. Coleman, B. and V.V.GLAVIANO. Tissue levels of norepinephrine and epinephrine in hemorrhagic shock. Science 139:54, 1963.
2. GLAVIANO, V.V. and B. Coleman. Effects of norepinephrine on myocardial electrolytes in hemorrhagic shock. Proc. Soc. Exp. Biol. and Med. 116:136-140, 1964.
3. Coleman, B. and V.V.GLAVIANO. Electrolyte and water distribution in the heart in irreversible hemorrhagic shock. Am. J. Physiol. 207:352-356, 1964.
4. GLAVIANO, V.V. Distribution and gradient of lactate between blood and heart muscle. Proc. Soc. Exp. Biol. and Med. 118:1155-1158, 1965.
5. GLAVIANO, V.V. and M. Klouda. Myocardial catecholamines and stimulation of the stellate ganglion in hemorrhagic shock. Am. J. Physiol. 209:751-756, 1965.
6. Yo, S. and V.V.GLAVIANO. Circulatory responses to bilateral common artery occlusion in hemorrhagic shock. Jap. J. Circ. 30:907, 1966 (English summary on p.899).
7. Doersching, J. and V.V.GLAVIANO. Glycogenolysis and phosphorylase activity of cardiac muscle in hemorrhagic shock. Am. J. Physiol. 211:1451-1454, 1966.
8. GLAVIANO, V.V. and S. Yo. Baroreceptor reflex activity in hemorrhagic shock. in International Symposium on Baroreceptors and Hypertension. Oxford: Pergamon Press, 1967.
9. GLAVIANO, V.V., S. Yo and T. Masters. Levels of lactate in lung tissue during sympathetic stimulation. Am. J. Physiol. 213:437-440, 1967.
10. Cova, R. and V.V.GLAVIANO. Myocardial and splenic levels of catecholamines in burn shock. Proc. Soc. Exp. Biol. and Med. 128:642-645, 1968.
11. GLAVIANO, V.V., A. Suzuki and R. Cova. Uptake and storage of lung histamine in burn shock. Proc. Soc. Exp. Biol. and Med. 130:387-390, 1969.
12. Masters, T.N. and V.V.GLAVIANO. Effects of dl-propranolol on myocardial carbohydrate and free fatty acid metabolism. J. Pharmac. Exp. Therap. 167:187-193, 1969.
13. GLAVIANO, V.V. and T. N. Masters. Effect of norepinephrine on cardiac metabolism before and after beta-receptor blockade. European J. Pharmacol. 7:135-142, 1969.
14. Suzuki, A. and V.V.GLAVIANO. A comparison between the pulmonary A-V difference of histamine and the severity of burn injury. Kobe J. Med. Sci. 15:159-162, 1969.
15. GLAVIANO, V.V. and T. N. Masters. Inhibitory action of intracoronary prostaglandin E_1 on myocardial lipolysis. Am. J. Physiol. 220:1187-1192, 1971.
16. Okamoto, R., V.V.GLAVIANO and M. Pindok. Myocardial lipases and catecholamines in burn shock. Proc. Soc. Exp. Biol. and Med. 137:347-353, 1971.
17. Suzuki, A., H. Matsumoto, N. Ito and V.V.GLAVIANO. Influence of thermal injury on the histamine levels of skin and blood. Kobe J. of Med. Sci. 17:59-63, 1971.

18. Masters, T.N. and V.V.GLAVIANO. Subcellular alterations in myocardial lipid metabolism in response to dl-propranolol and norepinephrine. J. Pharmacol. Exp. Therap. 182:246-255, 1972.
19. Deets, D. and V.V.GLAVIANO. Plasma and myocardial arteriovenous difference of lactic dehydrogenase activity in acute burn trauma. Proc. Soc. Exp. Biol. and Med. 142:412-416, 1973.
20. Coldberg, J.M., and V.V.GLAVIANO. The adrenals as a site of action for carbachol induced increases in plasma free fatty acids and glucose. Arch. Int. Pharmacodyn. Ther. 206:338-344, 1973.
21. Okamoto, A., M. Kaye, B. Coleman and V.V.GLAVIANO. Hemodynamic and metabolic alterations of the heart in burn shock. Circulatory Shock 1:243-250, 1974.
22. Coleman, B., J. E. Kallal, L. P. Feigen and V.V.GLAVIANO. Myocardial performance during hemorrhagic shock in the pancreatectomized dog. Am. J. Physiol. 228:1462-1468, 1975.
23. GLAVIANO, V.V., J. Goldberg and M. Pindok. Acetylcholine and norepinephrine interactions on cardiac lipids and hemodynamics. Am.J.Physiol. 228:1678-1684, 1975.
24. Feigen, L.P., B. Coleman, and V.V.GLAVIANO. Effects of alpha-adrenergic blockade on renal function in hemorrhagic shock. Am. J. Physiol. 232:F409-F415, 1977.